Primordial Germ Cells in Mice

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SUMMARY

Germ cell development creates totipotency through genetic as well as epigenetic regulation of the genome function. Primordial germ cells (PGCs) are the first germ cell population established during development and are immediate precursors for both the oocytes and spermatogonia. We here summarize recent findings regarding the mechanism of PGC development in mice. We focus on the transcriptional and signaling mechanism for PGC specification, potential pluripotency, and epigenetic reprogramming in PGCs and strategies for the reconstitution of germ cell development using pluripotent stem cells in culture. Continued studies on germ cell development may lead to the generation of totipotency in vitro, which should have a profound influence on biological science as well as on medicine.

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1 INTRODUCTION

In most multicellular organisms, including mammals, germ cells are the origin of new organisms and ensure the perpetuation of the genetic and epigenetic information across the generations. Accordingly, they prepare for totipotency during their ontogeny through genetic and epigenetic regulations of their genome function (Fig. 1).

There are several key properties/critical themes commonly associated with germ cell development in animals. Generally, the germ cell lineage is set aside from the somatic lineages early in development, either through localized maternal determinants (most typically referred to as germ plasm) or induction from pluripotent embryonic cells (Box 1). In many animals, primordial germ cells (PGCs) are the first germline cell population (Saitou and Yamaji 2010), which colonize the developing gonads by active migration (Richardson and Lehmann 2010). In the gonads, PGCs initiate differentiation either toward a spermatogenic (male) or an oogenic (female) pathway. The spermatogenic pathway most typically involves the establishment of spermatogonial stem cells (or male germline stem cells [GSCs]) with an enormous mitotic potential, whereas it appears that female GSCs in adults are rare across the phylogenetic spectrum (Spradling et al. 2011): *Caenorhabditis elegans*, *Drosophila*, and medaka fish (Nakamura et al. 2010) seem

to be among the rare examples of female GSCs in adults. In other animals, including mammals, the oogenesis is dependent on PGCs set aside in development, although some cells in the adult ovary may retain germline potential (White et al. 2012).

In this article, we focus on recent advances in the study of PGCs in mice on the mechanisms of PGC specification, potential pluripotency, and genome-wide epigenetic reprogramming in PGCs, as well as the induction of PGC fate in culture. Related themes, such as the properties of germline stem cells and small RNA-based genome defense mechanisms, have been reviewed elsewhere (Malone and Hannon 2009; Saito and Siomi 2010; Spradling et al. 2011).

2 GERM CELL DEVELOPMENT IN MICE

In the mouse, PGCs first become identifiable as a cluster of approximately 40 cells at the base of the incipient allantois at around embryonic day 7.25 (~E7.25) (Figs. 1 and 2) (Chiquoine 1954; Ginsburg et al. 1990). They migrate to the developing hindgut endoderm at ~E7.75, into the mesentery at ~E9.5, and colonize the genital ridges at ~E10.5 (Tam and Snow 1981; Molyneaux et al. 2001; Seki et al. 2007; Richardson and Lehmann 2010). A key event that occurs in PGCs during this proliferative phase both in the male and female is epigenetic reprogramming—most

Figure 1. A schematic representation of germ cell development in mice. A brief outline of germ cell development in mice is shown schematically. Key events associated with each stage of germ cell development are also shown. (Left) PGC development (from E6.25 to ~E12.5); (upper right) male germ cell development (from ~E13.5); (lower right) female germ cell development (from ~E13.5).

BOX 1. PREFORMATION VERSUS EPIGENESIS IN GERM CELL SPECIFICATION

There are two distinct mechanisms for the specification of the germ cell lineage: One is through the inheritance of localized determinants, often called the germ plasm, in the egg (pre-formation), and the other is through the induction from pluripotent embryonic cells (epigenesis) (Extavour and Akam 2003). The former mode is seen in many model organisms, including *C. elegans*, *D. melanogaster*, *Danio rerio*, and *Xenopus laevis*, whereas the latter mode is seen typically in mammals. Evolutionarily, the latter epigenesis mode appears to be more prevalent and ancestral to the metazoa, whereas the pre-formation mode appears to have arisen independently at multiple times, probably because of its better ability to secure the germline at the very onset of development (Extavour and Akam 2003).

Although the mechanisms for the segregation of the germ cell fate are divergent, some of the properties that the specified PGCs are endowed with appear to be conserved. One such property is the repression in PGCs of the somatic differentiation program robustly activated in surrounding somatic cells. In *C. elegans* and *D. melanogaster*, this is achieved through transient global repression of the RNA polymerase II (Pol II) activity by PIE-1 and the polar granule component, respectively (Nakamura et al. 1996; Seydoux et al. 1996). PIE-1, bearing two CCH RNA-binding domains but showing no similarity to known transcriptional repressors, inhibits the transcriptional initiation and elongation activity of RNAPII by distinct mechanisms (Ghosh and Seydoux 2008). As a mechanism to inhibit transcriptional elongation, PIE-1 appears to inhibit the positive transcriptional elongation factor b (P-TEFb), which promotes the transcriptional elongation activity of RNAPII by phosphorylating the Ser-2 of the carboxy-terminal domain (CTD) of RNAPII (Zhang et al. 2003). On the other hand, the polar granule component, which is a short, 71-amino-acid protein conserved only among *Drosophila* species, inhibits the recruitment of P-TEFb to transcriptional sites (Hanyu-Nakamura et al. 2008). In contrast, in mice, germ cell specification requires active transcription, but the transcriptional repressor BLIMP1 specifically shuts off gene expression for a somatic mesodermal program (Kurimoto et al. 2008) (see main text).

notably, a genome-wide DNA demethylation that includes the erasure of genomic imprinting (Fig. 1) (Saitou et al. 2012).

In the female XX embryo, the PGCs continue to proliferate until ~E13.5 (when they reach around 25,000 in number) and subsequently enter into the prophase I of meiotic divisions (Hilscher et al. 1974; Speed 1982). Subsequently, they are arrested at the diplotene stage of prophase I of meiosis. Upon hormonal stimulation, oocytes complete the first meiotic division with concomitant extrusion of the first polar body. At the fertilization with a haploid spermatozoon, the oocyte completes the second meiotic division and extrudes the second polar body. In contrast to those in the female, XY PGCs enter into mitotic arrest upon entry into the genital ridges, and stay quiescent in the G₀/G₁ phase of the cell cycle for the remaining embryonic period (Hilscher et al. 1974; Western et al. 2008). Around day 5 postpartum (P5), many of them resume active proliferation, while some are recruited as spermatogonial stem cells (SSCs) (Fig. 1) (Russell 1990; Yoshida 2010; Spradling et al. 2011). In culture, germline stem cells (GSCs) bearing the abilities for long-term proliferation and for spermatogenesis upon transplantation into testes are established in the presence of GDNF (glial cell–derived neurotrophic factor), most readily from neonatal testes (Kanatsu-Shinohara et al. 2003). Thus, compared with the very limited size of the oocyte pools, the spermatozoa can be supplied essentially infinitely by the SSC system.

3 MECHANISM FOR PGC SPECIFICATION**3.1 Gene Expression Dynamics during PGC Specification**

A single-cell analysis of gene expression of the founder population of PGCs at E7.25 identified two genes, *Fragilis* and *Stella*, that are highly and specifically, respectively, expressed in PGCs (Saitou et al. 2002). *Fragilis* (also known as mouse interferon-induced protein like gene-1, *mil-1*/interferon-induced transmembrane protein 3, *Ifitm3*) (Tanaka and Matsui 2002) is a member of the interferon-inducible transmembrane proteins, whereas *Stella* (also known as Primordial germ cell 7, *Pgc7*/developmental pluripotency-associated 3, *Dppa3*) (Sato et al. 2002) is a small, highly basic nucleo–cytoplasmic shuttling protein. *Fragilis* begins to show expression around the most proximal epiblast cells at ~E6.25–E6.5, and its expression intensifies in the posterior extra-embryonic mesoderm, where AP-positive PGCs arise at ~E7.0–E7.25. *Stella* begins to express specifically in *Fragilis*-expressing cells in the extra-embryonic mesoderm at ~E7.0–E7.25 and continues to be expressed in migrating PGCs. The *Stella*-positive cells show high expression of tissue nonspecific alkaline phosphatase (*Tnap*), a gene for AP activity of PGCs (MacGregor et al. 1995). Cells with positivity for *Stella* and high levels of *Fragilis* repress the expression of Homeobox genes such as *Hoxb1* and *Hoxa1*, whereas *Fragilis*-positive but *Stella*-negative cells retain Hox gene expression (Saitou et al.

Figure 2. A schematic representation of PGC development in mice. A brief outline of PGC specification, migration, and colonization of the gonads in mice is shown schematically. (Green) *Blimp1*- and *Prdm14*-positive cells that appear in the most proximal epiblasts. (Lower middle panel) A view of the transverse section of the position indicated by a red bar in the lower left panel. Epi, epiblast; (AVE, anterior visceral endoderm; ExE, extra-embryonic ectoderm; PGCs, primordial germ cells; EM, embryonic mesoderm; ExM, extra-embryonic mesoderm; Hg, hindgut; DA, dorsal aorta; NT, neural tube; NoC, notochord; IM, intermediate mesoderm; NeC, nephrogenic cord.

2002). It has thus been proposed that the *Stella*-positive and Hox-negative cells are the established PGCs (Saitou et al. 2002). Gene-knockout studies, however, revealed that neither *Fragilis* nor *Stella* is essential for PGC specification (Payer et al. 2003; Lange et al. 2008). Instead, *Stella* has been found to be a critical factor to protect the maternal genome and paternally imprinted genes from genome-wide DNA demethylation that occurs in the zygotes (Nakamura et al. 2007), whereas *Fragilis* has a critical function in restricting the replication of multiple pathogenic viruses including influenza (Brass et al. 2009; Everitt et al. 2012).

Further screenings of the PGC transcriptome led to the identification of two key regulators for PGC specification (Fig. 3; See Table 1 for key genes affecting PGC specification and proliferation/survival): *Blimp1* (B-lymphocyte-induced maturation protein 1, also known as PR domain-containing

protein 1 [*Prdm1*]) and *Prdm14* (PR domain-containing protein 14) (Ohinata et al. 2005; Vincent et al. 2005; Yabuta et al. 2006; Kurimoto et al. 2008; Yamaji et al. 2008). BLIMP1 and PRDM14 are evolutionarily conserved proteins. Together with *Tcfap2c* (also known as *AP2γ*) (Weber et al. 2010), these genes are found to be required for PGC specification in mammals.

Blimp1-positive cells first appear in a few of the most proximal epiblast cells at the posterior side of the embryo ~E6.25 (pre-primitive streak/no streak [P/OS] stage) (Fig. 2). They increase in number and form a cluster of cells with strong AP activity, and repression of *Stella* and the Hox gene (Ohinata et al. 2005). *Prdm14* is first expressed at ~E6.5 (early streak [ES] stage) in *Blimp1*-positive cells and later in the PGCs (Yamaji et al. 2008). Although activation of *Prdm14* is independent of *Blimp1* (Yamaji et al. 2008), the expression of *Tcfap2c* at ~E6.75 (late streak/no

Figure 3. Transcriptional regulation of PGC specification. A potential genetic network for PGC specification is shown schematically. Arrows and lines with terminal bars indicate genetic pathways for activation and for repression, respectively, as demonstrated by *in vivo* experiments (Kurimoto et al. 2008; Yamaji et al. 2008). Dotted arrows and dotted lines with terminal bars indicate genetic pathways for activation and for repression, respectively, as proposed based on *in vitro* experiments (Covello et al. 2006; West et al. 2009; Weber et al. 2010).

allantoic bud [LS/0B] stage) appears to be dependent on *Blimp1* (Kurimoto et al. 2008).

All the *Blimp1*-positive PGC precursors initially express the Hox genes and repress *Sox2* (Yabuta et al. 2006; Kurimoto et al. 2008). However, from E6.75 onward, *Blimp1*-positive cells begin to repress the Hox genes and express *Sox2*, *Stella*, and *Nanog* (Yamaguchi et al. 2005; Kurimoto et al. 2008). *Blimp1*-positive cells continue to express *Oct4* (also known as *Pou5f1*) PGCs at E7.25 up-regulate and down-regulate about 500 “germ cell specification” genes and around 330 “somatic program” genes, respectively (Kurimoto et al. 2008). Although *Blimp1*-positive PGCs delaminate from the epiblast, they do not display typical epithelial–mesenchymal transition (Kurimoto et al. 2008).

3.2 The Functions of *Blimp1*, *Prdm14*, and *Tafap2c*

BLIMP1 is a transcriptional repressor bearing a PR (PRDI-BF1 and RIZ) domain at its amino terminus and five Krüppel-type zinc fingers at its carboxyl terminus (Turner

et al. 1994). The PR domain is structurally similar to the SET (Suppressor of hairless, Enhancer of zeste, and Trithorax) domain, which is known to show histone methyltransferase activity (Rea et al. 2000). The PR domain of BLIMP1 has not yet been shown to exhibit enzymatic activity. BLIMP1 interacts with many distinct epigenetic regulators, including HDACs (Yu et al. 2000), Groucho (Ren et al. 1999), and G9A (Gyory et al. 2004), in a context-dependent manner (Bikoff et al. 2009).

In *Blimp1*-knockout embryos, AP-positive PGC-like cells appear at around the E/MB stage, but they are smaller in number, do not show a migratory phenotype, and are unable to express PGC-specific genes properly (Table 1) (Ohinata et al. 2005; Vincent et al. 2005). A single-cell cDNA microarray analysis has shown that, in the absence of *Blimp1*, nearly all of the somatic genes fail to be repressed, whereas approximately half of the specification genes are up-regulated normally (Kurimoto et al. 2008). Therefore, primarily, BLIMP1 is a potent transcriptional repressor responsible for the repression of the somatic genes. The

Table 1. Key genes affecting PGC specification and proliferation/survival

Gene	Function	Expression ^a	Loss-of-function phenotype in PGCs
PGC SPECIFICATION			
Cytokines			
<i>Bmp4</i>	A BMP/GDF/MIS subfamily ligand of the TGF- β family	ICM, ExE from E5.5, ExM during gastrulation	No and reduced number of PGCs in homozygous and heterozygous mutants, respectively; loss of <i>Blimp1</i> and <i>Prdm14</i> expression in homozygous mutants; loss of <i>Bmp4</i> in the ExM leading to abnormal PGC localization and survival (Lawson et al. 1999; Fujiwara et al. 2001; Yamaji et al. 2008; Ohinata et al. 2009)
<i>Bmp8b</i>	A BMP/GDF/MIS subfamily ligand of the TGF- β family	ExE from E5.5	No and reduced number of PGCs in homozygous and heterozygous mutants, respectively (C57BL/6 background); severely impaired <i>Blimp1</i> and <i>Prdm14</i> expression in homozygous mutants (Ying et al. 2000; Ohinata et al. 2009)
<i>Bmp2</i>	A BMP/GDF/MIS subfamily ligand of the TGF- β family	VE	Reduced numbers of PGCs both in the heterozygous and homozygous mutants (C57BL/6 background) (Ying and Zhao 2001)
<i>Wnt3</i>	A secreted glycoprotein of the Wnt family	Posterior proximal epiblast and VE, primitive streak, mesodermal wing	Loss of <i>Blimp1</i> and <i>Prdm14</i> expression in homozygous mutants (Ohinata et al. 2009)
Signal transducers			
<i>Smad1</i>	A receptor Smad for the BMP/GDF/MIS subfamily	Ubiquitous	No PGCs in homozygous mutants; severely impaired <i>Blimp1</i> and <i>Prdm14</i> expression in homozygous mutants (Tremblay et al. 2001; Hayashi et al. 2002; Aubin et al. 2004; Yamaji et al. 2008; Ohinata et al. 2009)
<i>Smad5</i>	A receptor Smad for the BMP/GDF/MIS subfamily	Ubiquitous	No and reduced number of PGCs in homozygous and heterozygous mutants, respectively (Chang and Matzuk 2001)
<i>Smad4</i>	A common partner for all receptor Smads	Ubiquitous	Severely reduced number of PGCs in epiblast-specific <i>Smad4</i> knockouts (Chu et al. 2004)
<i>Smad2</i>	A receptor Smad for the TGF- β /Activin/Nodal subfamily	Ubiquitous	Abundant PGCs at E8.5 in surviving embryos; widespread <i>Blimp1</i> expression in early gastrulating embryos (Ohinata et al. 2009)
<i>FoxH1</i>	A winged-helix transcription factor	Ubiquitous	Widespread <i>Blimp1</i> expression in early gastrulating embryos (Ohinata et al. 2009)
Receptors			
<i>Alk2</i>	A type I BMP receptor (serine/threonine kinase)	Primarily VE before gastrulation	No and reduced number of PGCs in homozygous and heterozygous mutants, respectively (de Sousa Lopes et al. 2004)
Transcription factors (TFs)			
<i>Blimp1</i>	A TF with PR domain and Zinc fingers	PGCs, VE, many other lineages	Impaired PGC specification; reduced number of PGCs in heterozygous mutants (Vincent et al. 2005; Ohinata et al. 2009)
<i>Prdm14</i>	ATF with PR domain and zinc fingers	PGCs, ICM	Impaired PGC specification (Yamaji et al. 2008)
<i>Tcfap2c</i>	A basic helix–span–helix TF	All cells until the blastocyst stage, ExE, PGCs	Impaired PGC specification in epiblast-specific <i>Tcfap2c</i> knockouts (Weber et al. 2010)
<i>Oct4</i>	A POU-homeobox domain TF	Ubiquitous in the embryo proper until E7.5, specific to germ cells thereafter	Impaired PGC specification; apoptosis of PGCs (Kehler et al. 2004; Okamura et al. 2008)
RNA-binding protein			
<i>Lin28</i>	An RNA-binding protein	Ubiquitous	Reduced number of PGCs by RNAi-mediated <i>Lin28</i> knockdown (West et al. 2009)

Continued

Table 1. Continued

Gene	Function	Expression ^a	Loss-of-function phenotype in PGCs
Cell adhesion molecules			
<i>Cdh1</i>	A homophilic cell–cell adhesion molecule	Epiblast, PGCs, and their somatic neighbors	An inhibitory antibody blocking PGC specification (Okamura et al. 2003)
PGC PROLIFERATION/SURVIVAL			
Cytokines			
<i>Scf</i>	A short-chain helical cytokine	The migratory path of PGCs	Impaired proliferation/survival and migration of PGCs (McCoshen and McCallion 1975; Gu et al. 2009)
<i>Cxcl12</i>	A chemokine ligand	Gonad and subjacent mesonephric tissue at E12.5, widespread in embryos	Impaired migration of PGCs (Ara et al. 2003; Molyneaux et al. 2003)
<i>Fgf9</i>	An FGF family ligand	Specific to male gonads after E12.5	Impaired survival of male PGCs after E11.5 (DiNapoli et al. 2006)
<i>Bmp7</i>	A BMP/GDF/MIS subfamily ligand of the TGF- β family	Specific to male gonads after E12.5	Impaired PGC proliferation during E10.5–E11.5 in males (Ross et al. 2007)
<i>Wnt5a</i>	A secreted glycoprotein of the Wnt family	Widespread, up-regulated in testicular interstitial cells after E13.5	Impaired proliferation/survival and migration of PGCs (Chawengsaksophak et al. 2011; Laird et al. 2011)
Signal transducers			
<i>Pten</i>	A lipid phosphatase	Ubiquitous	Bilateral testicular teratoma (Kimura et al. 2003)
<i>Bax</i>	A proapoptotic factor of the Bcl2 family	Ubiquitous	Enhanced PGC survival (Stallock et al. 2003; Runyan et al. 2006; Suzuki et al. 2008; Cook et al. 2009; Gu et al. 2009)
Receptors			
<i>Kit</i>	A receptor tyrosine kinase	PGCs	Impaired proliferation/survival and migration of PGCs (Mintz and Russell 1957; Buehr et al. 1993)
<i>Cxcr4</i>	A seven-transmembrane spanning G-protein-coupled receptor	PGCs	Impaired migration/survival of PGCs (Ara et al. 2003; Molyneaux et al. 2003)
<i>Fgfr2-IIIb</i>	A receptor tyrosine kinase	Widespread	Impaired PGC survival at E11.5 (Takeuchi et al. 2005)
<i>Ror2</i>	A receptor tyrosine kinase	Widespread	Impaired proliferation/survival and migration of PGCs (Laird et al. 2011)
Transcription factors			
<i>Oct4</i>	A POU-homeobox domain TF	Ubiquitous in the embryo proper until E7.5, specific to germ cells thereafter	Impaired PGC specification; apoptosis of PGCs (Kehler et al. 2004; Okamura et al. 2008)
<i>Nanog</i>	A divergent homeodomain TF	Morula, ICM, epiblast, primitive streak, PGCs	Apoptosis of PGCs (Chambers et al. 2007; Yamaguchi et al. 2009)
<i>Hif2α</i>	A bHLH-PAS TF	Ubiquitous	Reduced number of PGCs at E8.5 and eventual loss of PGCs (Covello et al. 2006)
RNA-binding proteins			
<i>Nanos3</i>	An RNA-binding protein	PGCs	Reduced number of PGCs at E8.0 and eventual loss of PGCs (Tsuda et al. 2003; Suzuki et al. 2008)
<i>Dnd1</i>	An RNA-binding protein	PGCs, widespread in embryos at E7.5	Reduced number of PGCs at E8.0 and eventual loss of PGCs (Sakurai 1995; Youngren et al. 2005; Cook et al. 2009)
<i>Tiar</i>	An RNA-binding protein	Ubiquitous	Reduced number of PGCs in the genital ridges at E11.5 and eventual loss of PGCs (Beck et al. 1998)
<i>Mvh</i>	A DEAD-box RNA helicase	PGCs after E10.5	Impaired male PGC proliferation after E11.5 (Tanaka et al. 2000)
<i>Dicer</i>	RNase	Ubiquitous	Impaired PGC proliferation (Hayashi et al. 2008)
miRNAs			
<i>Mir-290-295</i>	Posttranscriptional regulation of gene expression	ESCs, embryos before E6.5, PGCs	Impaired migration/survival of PGCs (Medeiros et al. 2011)

Continued

Table 1. *Continued*

Gene	Function	Expression ^a	Loss-of-function phenotype in PGCs
Cell adhesion molecules			
<i>Gja1</i>	A gap-junction protein	PGCs, widespread in embryos	Impaired colonization of PGCs (Juneja et al. 1999; Francis and Lo 2006)
<i>Itgb1</i>	A β subunit of integrin complex	PGCs, widespread in embryos	Impaired migration of PGCs (Anderson et al. 1999)
Others			
<i>Hpl1γ</i>	Heterochromatin protein with binding to H3K9me	Ubiquitous	Reduced number of PGCs after E7.25 due to impaired cell cycle (Abe et al. 2011)
<i>Mcm9</i>	A minichromosome maintenance helicase paralog	Ubiquitous	Impaired PGC proliferation (Hartford et al. 2011)
<i>Ft locus</i>	A region of mouse chromosome 8 bearing six genes	Detected in embryonic gonads	Impaired PGC proliferation (Kim et al. 2011)

^aTissue types: ESC, embryonic stem cell; ExE, extra-embryonic ectoderm; ExM, extra-embryonic mesoderm; ICM, inner cell mass; PGC, primordial germ cell; VE, visceral endoderm.

BMP4-SMAD1/5 signaling pathway may mediate the BLIMP1-independent acquisition of the specification genes (Fig. 3) (see below). The precise mechanism of action of BLIMP1 in PGC specification remains to be determined.

PRDM14 also bears the PR-domain and five Krüppel-type zinc fingers. *Prdm14* is transiently expressed in the ICM and later only in the germline until $\sim$ E13.5 (Yamaji et al. 2008). *Prdm14* is essential for PGC specification (Table 1). *Prdm14*-deficient cells fail to reactivate *Sox2*, although they repress the somatic mesodermal program normally. They also fail to repress GLP (also known as EHMT1), an enzyme essential for the genome-wide H3K9me2 modification (Tachibana et al. 2005), and fail to reduce and up-regulate their H3K9me2 and H3K27me3 levels, respectively, for the acquisition of pluripotency and genome-wide epigenetic reprogramming (Fig. 3). *Prdm14*-deficient cells cannot form embryonic germ cells (EGCs) in culture. *Prdm14* may act as a sequence-specific transcriptional regulator and is essential for the pluripotency of mice and human embryonic stem (ES) cells (Tsuneyoshi et al. 2008; Chia et al. 2010; Ma et al. 2011).

TCFAP2C (AP2 γ) is a basic helix–span–helix domain transcription factor. It shows maternal expression in all cells until the blastocyst stage, but its expression is restricted in ExE and PGCs from E6.75 up to E12.5–E13.5 (Auman et al. 2002; Werling and Schorle 2002; Weber et al. 2010). Epiblast-specific deletion of *Tcfap2c* results in the drastically reduced PGCs, which show little migration and are lost later (Table 1) (Weber et al. 2010). TCFAP2C may function downstream from BLIMP1 in PGCs (Fig. 3).

3.3 Signaling for PGC Specification

Understanding the signaling requirements for the induction of PGCs from the epiblasts is of critical importance to

develop a culture system to recapitulate PGC specification in culture. An integrated view of the signaling pathways for PGC specification has been proposed based on genetic as well as in vitro culture experiments that examined the influence of signaling molecules on the induction of *Blimp1* and *Prdm14* expression in the epiblast (Fig. 3) (Ohinata et al. 2009).

Accordingly, bone morphogenetic protein 4 (BMP4) emitted from the extra-embryonic ectoderm (ExE) activates the expression of *Blimp1* and *Prdm14* in a dose-dependent manner. BMP2 expressed in the proximal VE enhances the same signaling pathway, ensuring that the highest levels of BMP signaling occur in the most proximal epiblast. These findings are consistent with the previous observations that PGC specification has been shown to depend on the dosage of BMP signaling and the level of *Blimp1* induction (Lawson et al. 1999; Ohinata et al. 2005; Vincent et al. 2005), and that mutants for *Bmp4*, *Bmp8b*, *Bmp2*, *Smad1*, *Smad4*, *Smad5*, and *Alk2* lack or have reduced numbers of AP-positive PGCs (Table 1) (Zhao 2003; Saitou and Yamaji 2010). The induction of *Blimp1* expression in the posterior side of the embryo is explained by the inhibition of BMP signaling in the anterior epiblast by antagonist factors (e.g., LEFTY1 against NODAL, DKK1 against WNT, and Cerberus-like [CER1] against BMP, etc.) that are emanated from the anterior visceral endoderm (AVE) (Umulis et al. 2009). In *Smad2* or *Foxh1* mutants that fail to form AVE, *Blimp1* expression becomes widespread in the epiblast. Epiblast that is striped of the AVE also express *Blimp1* and *Prdm14* in response to BMP4.

WNT signaling is also essential for the PGC fate (Table 1): *Wnt3*-knockout epiblast cells do not show *Blimp1* expression in response to BMP4 (Ohinata et al. 2009). *Wnt3* is expressed in the epiblast and VE from $\sim$ E5.5 (Liu et al. 1999), and epiblast cells of embryos younger than this

age do not express *Blimp1* in response to BMP4 (Ohinata et al. 2009). *Wnt3* may therefore prime the epiblasts to express *Blimp1* in response to BMP4. *Wnt3*-knockout embryos express receptors (*Alk3*, *Alk6*, *Bmpr1I*) and signal transducers (*Smad1*, *Smad5*) for BMP signaling (Ohinata et al. 2009), suggesting that the WNT signaling may act posttranscriptionally on BMP signaling components. In this regard, WNT signaling can stabilize SMAD1 by inhibiting GSK3-mediated phosphorylation of its linker region and prevents its degradation (Fuentelba et al. 2007; Sapkota et al. 2007).

Significantly, most, if not all, of the epiblast cells cultured under a serum-free, defined condition primarily with BMP4 show *Blimp1* and *Prdm14* after 36 h, and these cells go on to develop *Blimp1*-, *Prdm14*-, and *Stella*-positive PGC-like cells after 132 h (Ohinata et al. 2009). They not only show proper gene expression like that of the PGCs, but also show genome-wide epigenetic reprogramming, including H3K9me2 reduction, H3K27me3 up-regulation, and erasure of an imprinted gene. When transplanted into the neonatal testes of germ-cell-less *W/W^v* mice, these cells contribute to proper spermatogenesis, and the resultant spermatozoa contribute to fertile offspring, a bona fide demonstration that the PGC-like cells induced from the epiblasts ex vivo acquire the germ cell phenotype (Ohinata et al. 2009). These findings provide a basis for generating the germ cell lineage in mammals in vitro.

4 PGCs FROM PLURIPOTENT STEM CELLS

The ability to generate PGCs from epiblast explants points to an effective strategy to produce functional PGCs from pluripotent stem cells (e.g., embryonic stem cells [ESCs], induced pluripotent stem cells [iPSCs]) in vitro by directed differentiation (Fig. 4) (Hayashi et al. 2011). The functional attributes of these in vitro–derived germline cells may be tested as for spermatogenesis potential by transplanting into neonatal testes lacking endogenous germ cells (Chuma et al. 2005), and for oogenesis potential by aggregating with gonadal somatic cells and transplanted under kidney corpuscles to allow oocyte growth and maturation (Matoba and Ogura 2011).

Several critical milestones of germ cell development have to be recapitulated during in vitro differentiation of ESCs. First, ESCs have to be induced into epiblast-like cells (EpiLCs) by treatment with ActivinA (ACTA), bFGF, and a low concentration of KSR (Fig. 4) (Hayashi et al. 2011). The EpiLCs are a transient entity and show a global gene expression profile similar to that of the pre-gastrulating epiblasts at E5.75, but distinct from that of epiblast stem cells (EpiSCs) (Brons et al. 2007; Tesar et al. 2007; Hayashi et al. 2011): For example, EpiSCs express the key pluripotency

Figure 4. The generation of PGC-like cells from ESCs/iPSCs in culture. A schematic representation of the generation of PGC-like cells (PGCLCs) from ESCs/iPSCs in culture (Hayashi et al. 2011). ESCs/iPSCs are induced into epiblast-like cells (EpiLCs) by the stimulation with ActivinA, bFGF, and KSR (knockout serum replacement) for 2 d. EpiLCs (about 1000 cells) are aggregated in a floating condition and are induced into PGCLCs by the stimulation with BMP4, BMP8b, SCF (stem cell factor), LIF (leukemia inhibitory factor), and EGF (epidermal growth factor). *Blimp1*-positive PGCLCs (green) are induced and form clusters at peripheries of the aggregate. PGCLCs are sorted by FACS (fluorescence activated cell sorting) according to their expression of *Blimp1* transgene, or of surface markers, SSEA1 (stage-specific embryonic antigen 1), and Integrinβ3. PGCLCs are transplanted into neonatal testes lacking endogenous germ cells, leading to proper spermatogenesis. The ESC/iPSC-derived sperm are injected into oocytes by ICSI (intracytoplasmic sperm injection), and the resultant two-cell embryos are transferred to pseudopregnant females, resulting in the generation of fertile offspring.

gene *Nanog* at a high level, but EpiLCs and the epiblasts at E5.75 express it at a low level. EpiSCs also express endoderm markers such as *Gata6*, *Sox17*, and *Cer1* at a high level, but EpiLCs and the epiblasts at E5.75 express them at a low/undetectable level (Hayashi et al. 2011). Remarkably, under conditions that induce the epiblast into PGC-like cells ex vivo, EpiLCs produce *Blimp1*-, *Prdm14*-, and

Stella-positive PGC-like cells (PGCLCs), whereas EpiSCs show some *Blimp1* but not *Stella* expression (Hayashi et al. 2011). The PGCLCs show a global gene expression profile very similar to that of PGCs at E9.5 (Hayashi et al. 2011). The PGCLCs show genome-wide epigenetic reprogramming (reduction of H3K9me2 and elevation of H3K27me3) and undergo spermatogenesis when transplanted into the testes of neonatal *W/W^v* mice, and the resultant sperm contribute to healthy, fertile offspring (Fig. 4) (Hayashi et al. 2011). The induction of EpiLCs and PGCLCs can be achieved in many ESC and iPSCs (Fig. 4) (Hayashi et al. 2011).

One of the key challenges for the generation of the stem cell–derived gametes in vitro is the sustenance of the proliferation of PGCLCs or PGCs to expand these germline precursor cells, in the same manner as between E9.5 and E12.5 in vivo, when their numbers increase from approximately 250 to 25,000 (Tam and Snow 1981). PGCLCs are short-lived (Hayashi et al. 2011), and PGCs in culture undergo apoptosis after several rounds of cell division (Dolci et al. 1991; Matsui et al. 1991; Pesce et al. 1993; Kawase et al. 1996) or dedifferentiate into EGCs (Matsui et al. 1992; Labosky et al. 1994).

Efficient PGCLC induction in mice depends strictly on the use of ESCs cultured under a serum-free condition with an inhibitor against MAP kinase signaling, an inhibitor against GSK3, and LIF (2i + LIF), a condition that maintains ESCs in an undifferentiated self-renewing “ground state” (Ying et al. 2008) and the derivation of EpiLCs as the immediate precursor for PGCLCs (Hayashi et al. 2011). So far, this has not been accomplished in human ESCs. That EpiSCs are functionally different from pre-gastrulating epiblast cells in germ-cell-forming capacity has significant implications for germ cell differentiation from human ESCs, which are believed to closely resemble EpiSCs (Nichols and Smith 2009). The different properties between mouse and non-rodent mammalian ESCs may be a reflection of the presence of different molecular logic in the mechanisms for early development between mouse and non-rodent mammals. It is also of note that the modes of gastrulation, during which PGCs emerge, are different between mice and non-rodent mammals, including humans (Sadler 2010; Hopf et al. 2011). It is therefore important to elucidate the process of germ cell specification in other non-rodent mammals that develop similarly to humans, before any rational approach can be devised for differentiating hESCs/iPSCs into germ cells.

5 REVEALING PLURIPOTENCY IN PGCs

PGCs have long been known as origins for teratoma/teratocarcinoma (Stevens 1964). The investigation of teratoma/

teratocarcinomas and the stem cells derived from them (embryonal carcinoma cells, ECCs) eventually led to the derivation of ESCs from the ICM in culture (Evans and Kaufman 1981). The mechanism underlying cellular pluripotency has been studied most extensively using ESCs. The pluripotency is regulated and stably maintained mainly by a network of the core transcriptional regulators OCT4, SOX2, and NANOG (Niwa 2007; Young 2011). These transcription factors are expressed in the ICM and early epiblasts, but upon epiblast differentiation, although OCT4 remains expressed until relatively late, SOX2 and NANOG are down-regulated, especially in the posterior regions. As discussed above, PGCs, although essentially unipotent in vivo, reacquire the network of key genes for pluripotency upon their specification: PGCs continue to express OCT4 and regain the expression of SOX2 and NANOG (Fig. 3).

5.1 The Functions of Pluripotency Genes in PGCs

There is evidence that *Oct4* is essential for the emergence of *Stella*-positive PGCs (Table 1) (Okamura et al. 2008). Conditional knockout of *Oct4* or conditional knockdown of *Nanog* in migrating PGCs results in the death of PGCs by apoptosis, indicating the importance of these gene products in the maintenance of PGCs (Table 1) (Kehler et al. 2004; Chambers et al. 2007; Yamaguchi et al. 2009). Hypoxia-inducible factor 2α (HIF2α), which has a function to directly up-regulate *Oct4*, is also critical in PGC specification and/or survival (Fig. 3; Table 1) (Covello et al. 2006). Furthermore, LIN28, a negative regulator of *let-7* microRNA (miRNA, mir) family processing and a key factor for pluripotency (Gangaraju and Lin 2009), appears to be essential for PGC specification (West et al. 2009). Because *let-7* is known to inhibit *Blimp1* mRNA translation by binding to its 3′ UTR (Nie et al. 2008), it is proposed that LIN28 activates *Blimp1* by blocking the maturation of functional *let-7* (Fig. 3; Table 1) (West et al. 2009). *Mir-290-295*, which is expressed in early embryos, ESCs, and PGCs and facilitates induced pluripotency (Houbaviy et al. 2003; Marson et al. 2008; Judson et al. 2009), has also been shown to be essential for PGC development (Table 1) (Medeiros et al. 2011). Thus, the genes associated with pluripotency are critical in PGC specification and proliferation/survival. Among the core pluripotency factors, SOX2 is not present in human PGCs, indicating that a different mechanism may underpin the pluripotency between the mouse and the human (Perrett et al. 2008).

Upon specification, PGCs express essential factors required for induced pluripotency, including OCT4, SOX2, NANOG, LIN28, KLF2, KLF5, and N-MYC (Fig. 3) (Takahashi and Yamanaka 2006; Yu et al. 2007; Kurimoto et al.

2008; Nakagawa et al. 2008). A key difference between PGC specification and induced pluripotency would be that the PGC fate is induced in epiblast cells that already bear key attributes of pluripotency. PGCs reacquire robust expression of SOX2, NANOG, KLF2, KLF5, and N-MYC during their specification (Kurimoto et al. 2008). Furthermore, the inactivation of the p53 pathway enhances EGC formation from PGCs (see below) (Kimura et al. 2008). p53 is a major tumor suppressor and prevents tumor formation from damaged or stressed cells by triggering apoptosis, by activating cell cycle checkpoints, or by promoting permanent cell cycle arrest (Vousden and Prives 2009; Hanahan and Weinberg 2011). Notably, the formation of pluripotent stem cells from spermatogonial stem cells and the induction of pluripotency from somatic cells by defined factors are also enhanced by the lack of p53 function (Kanatani-Shinohara et al. 2004; Hong et al. 2009; Kawamura et al. 2009; Li et al. 2009; Marion et al. 2009; Utikal et al. 2009), indicating a common mechanism regulating the dedifferentiation of PGCs/spermatogonial stem cells into pluripotent stem cells and induced pluripotency.

5.2 From PGCs to EGCs

Reflecting their ability to acquire pluripotency relatively easily, PGCs have been shown to dedifferentiate into pluripotent EGCs in culture in the presence of basic FGF, SCF (stem cell factor, also known as Kit-ligand and *Kitl*) (provided by the SL⁴-m220 feeder cells, which bear the membrane-bound form of SCF), and LIF at a ratio of ~1% (Fig. 5) (Matsui et al. 1992; Labosky et al. 1994). PGCs convert into EGCs with the ability to contribute to chimeras within 10 d in culture, and the critical window for the requirement of bFGF is the first 24 h (Fig. 5) (Durcova-Hills et al. 2006; Durcova-Hills et al. 2008). A key effect of FGF is the repression of BLIMP1 that allows the activation of KLF4 and c-MYC (Fig. 5). LIF is required on days 2–4 to activate STAT3, a key transcription factor for pluripotency. Interestingly, trichostatin A (TSA), an inhibitor of histone deacetylases, can act as a substitute for the required bFGF. Conditional deletion of *Pten* or constitutive activation of AKT partly replaces the requirement of bFGF and enhances the dedifferentiation of PGCs to EGCs through the inactivation of the p53 pathway (Fig. 5) (Kimura et al. 2003, 2008).

EGCs can also be derived both in mice and rats by culturing PGCs under the 2i + LIF condition (Leitch et al. 2010), which also facilitates induced pluripotency from somatic cells (Takahashi and Yamanaka 2006; Silva et al. 2008). The ratio of EGC derivation becomes as high as ~10% when PGCs are first stimulated by bFGF and SCF and then transferred to a 2i + LIF condition. Moreover,

the culture under the simple 2i + LIF condition alone is sufficient for EGC derivation, although the efficiency decreases in the absence of bFGF (Leitch et al. 2010). It is of note that AKT signaling, which is a key for EGC derivation and a substitute for bFGF (Kimura et al. 2008), acts in part by inhibiting GSK3. Thus, the successful activation of multiple signaling pathways/underlying transcription factors converts PGCs into EGCs (Fig. 5).

6 EPIGENETIC REPROGRAMMING IN PGCS

PGCs show genome-wide DNA demethylation, which, by E13.5, leads to the erasure of genomic imprinting, reactivation of the inactivated X chromosome in females, and demethylation of the transposable/repetitive elements, resulting in the creation of an epigenetically “inert” genome (Monk et al. 1987; Kafri et al. 1992; Szabo and Mann 1995; Hajkova et al. 2002; Lee et al. 2002; Lane et al. 2003; Popp et al. 2010; Guibert et al. 2012; Saitou et al. 2012). Genome-wide methylation analyses of CpG dinucleotide sequences have revealed a significant hypomethylation of both male and female PGCs at E13.5 (Popp et al. 2010; Guibert et al. 2012). One study shows that the female PGCs bear a lower methylation level than the male PGCs (Popp et al. 2010), which may be an effect of their having two active X chromosomes because of their X-reactivation (Zvetkova et al. 2005). In PGCs, long terminal repeat (LTR) retrotransposon sequences such as intracisternal A particles (IAPs) are more resistant to demethylation, whereas genic, intergenic, and other transposon sequences are apparently globally demethylated (Popp et al. 2010; Guibert et al. 2012). The resistance of the LTR sequences to demethylation can cause *trans*-generational epigenetic inheritance in some cases (Daxinger and Whitelaw 2012).

Given that PGCs initially bear a genome-wide methylation level similar to that of their precursor epiblast cells (Guibert et al. 2012; Saitou et al. 2012), they should possess mechanisms for genome-wide DNA demethylation, which still remain poorly elucidated. In mammals, the mechanism of active DNA demethylation has long been elusive (Ooi and Bestor 2008; Wu and Zhang 2010; see Gehring et al. 2009 for information regarding the known mechanism of DNA demethylation involving 5-methylcytosine [5mC] demethylases in plants). Although a number of potential mechanisms have been proposed for active DNA demethylation in animals, none has yet been convincingly shown to be responsible for genome-wide DNA demethylation in PGCs (Saitou et al. 2012). For example, there is evidence that the cytosine deaminases AID and APOBEC1 can convert 5-methylcytosine (5mC) to T's by deamination, creating T:G mismatches, which would then become targets of glycosylases such as TDG (thymine DNA

Figure 5. Regulatory network for EGC derivation from PGCs. Key signaling mechanisms (A) and events for the first 10 d during the derivation of EGCs from PGCs (B) are shown schematically. (A) Arrows indicate activation, whereas blunted arrows show repression. (B, upper panel) The requirement of the signaling molecules; (lower panel) gene expression dynamics.

glycosylase) or MBD4 (methyl-CpG binding domain protein 4) for base-excision mismatch repair (BER) (Morgan et al. 2004). Indeed, it appears that in *Aid*^{-/-} PGCs, global DNA demethylation is attenuated to some degree (Popp et al. 2010). However, it is important to note that *Aid* and *Apobec1* are expressed at very low levels in PGCs (Hajkova et al. 2010) and that both *Aid*^{-/-} male and female mice are fertile, with the *Aid*^{-/-} mice being maintained over many generations by intercrosses between homozygotes (Muramatsu et al. 2000), indicating that AID plays little, if any, role in germ cell development.

Recent studies have shown that the TET (ten-eleven translocation) proteins (TET1, 2, 3), which are 2-oxoglutarate and Fe(II)-dependent dioxygenases, hydroxylate

5mC into 5-hydroxymethylcytosine (5hmC) (Kriaucionis and Heintz 2009; Tahiliani et al. 2009), and successively into 5-formylcytosine (5fC) and into 5-carboxylcytosine (5caC) (He et al. 2011; Ito et al. 2011; Pfaffeneder et al. 2011). The resultant 5hmC, 5fC, and 5caC may be converted into C passively during DNA replication or removed by a BER pathway (He et al. 2011; Ito et al. 2011). The TET proteins are therefore new candidate DNA demethylases in PGCs, where *Tet1* and, to a lesser extent, *Tet2*, but not *Tet3*, are expressed (Hajkova et al. 2010). Several studies have shown the roles of TET proteins, most typically the role of TET1 in ESCs (Ito et al. 2010; Ficz et al. 2011; Koh et al. 2011; Pastor et al. 2011; Song et al. 2011; Williams et al. 2011; Wu et al. 2011; Xu et al. 2011). Accordingly, one of the

functions of TET1 would be to remove aberrant stochastic DNA methylation from promoters with high or intermediate CpG contents, thereby regulating DNA methylation fidelity in ESCs (Williams et al. 2012). Furthermore, significantly, TET3, which is highly expressed in the oocytes, has been shown to be involved in the active genome-wide DNA demethylation from the paternal haploid genome in the zygotes (Gu et al. 2011; Iqbal et al. 2011; Wossidlo et al. 2011). Thus, it is of particular interest and importance to explore whether the TET proteins (TET1 and TET2) are, indeed, involved in the genome-wide DNA demethylation in PGCs and, if so, whether the BER pathway would follow the TET-mediated 5mC hydroxylation (Hajkova et al. 2010). However, it has more recently been shown that *Tet1*-knockout ESCs are pluripotent and contribute robustly to chimeras and the *Tet1*-knockout mice are viable and fertile, although their litter sizes are somewhat smaller than those of the wild types (Dawlaty et al. 2011). To further examine the function of TET proteins in germ cell development and DNA demethylation in PGCs, *Tet1* and *Tet2* double-knockout mice need to be analyzed.

Although there is as yet no convincing evidence for an enzyme-triggered, active DNA demethylation in PGCs, there is circumstantial evidence that DNA demethylation in PGCs is mediated by a DNA repair mechanism (Hajkova et al. 2008, 2010). Accordingly, PGCs at $\sim$ E11.5 appear to undergo a global reorganization of their chromatin state, which includes concomitant loss of linker histone H1, chromocenters (clusters of pericentric heterochromatin domains of several chromosomes) (Probst and Almouzni 2011), and various histone modification marks, along with a significant enlargement of nuclei. Evidence shows that components of the BER pathway such as XRCC1, APE1, and PARP1 are specifically concentrated, and XRCC1 is bound to chromatin in the nuclei of PGCs (Hajkova et al. 2010). It has thus been suggested that the global chromatin reorganization in PGCs is a consequence of the DNA demethylation mediated by a genome-wide BER, which results in a genome-wide histone replacement, possibly by the histone chaperone HIRA or NAP1 (nucleosome assembly protein 1) (Hajkova et al. 2008, 2010). This is a testable prediction and requires exploration by genetic experiments such as knockouts of relevant genes.

Although there is a perception that the genome-wide epigenetic reprogramming in PGCs occurs after their colonization of the gonads, at least a part of the reprogramming process starts much earlier, nearly concurrent with PGC specification, and proceeds during the PGC migration period (Seki et al. 2005, 2007; Guibert et al. 2012). The promoter DNA demethylation in migrating PGCs (E8.5–E9.5) appears to be global and influences genes of various biological functions (Guibert et al. 2012).

Furthermore, PGCs reduce their genome-wide H3K9me2 from $\sim$ E7.75, and nearly all of the PGCs show reduced H3K9me2 levels by E8.75. In contrast, they start to up-regulate their genome-wide H3K27me3 from $\sim$ E8.25, and by E9.5 nearly all of the PGCs show up-regulation of H3K27me3. Interestingly, although PGCs at E7.25 or after E9.75 show a cell cycle distribution similar to that of ESCs, indicative of their rapid proliferation, PGCs that are migrating in the hindgut endoderm from $\sim$ E7.75 to E8.75 show a unique cell cycle distribution, with nearly 60% of them being in the G₂ phase (Seki et al. 2007). Coincident with this period, PGCs appear to shut off their RNA polymerase II–dependent global transcription (Seki et al. 2007). Therefore, the histone modification reprogramming in migrating PGCs occurs when they are in the G₂ phase of the cell cycle and are transcriptionally quiescent, apparently in a progressive, cell-by-cell fashion.

The mechanism and the significance of the histone modification reprogramming in migrating PGCs have been unknown. It is, however, of note that PGCs repress GLP and G9A (also known as EHMT2), key histone lysine methyltransferases for H3K9me2 (Tachibana et al. 2002, 2005), from $\sim$ E7.75 and E9.5, respectively. The identification of the sequences from which H3K9me2 is removed and to which H3K27me3 is added should provide critical information to explore the function of these events. It would be interesting to examine whether the H3K27me3 in PGCs marks genes similar to those in ESCs (Bernstein et al. 2006; Barski et al. 2007; Mikkelsen et al. 2007; Pan et al. 2007; Zhao et al. 2007; Mohn et al. 2008). It is also critical to note that PGCs repress DNMT3A/3B and UHRF1 soon after their specification (Seki et al. 2005; Kurimoto et al. 2008) and therefore would bear limited *de novo* as well as maintenance methyltransferase activities during their development. The low H3K9me2, high H3K27me3, and limited DNA methyltransferase activities in PGCs may facilitate passive, rather than active, genome-wide DNA demethylation. Additionally, PGCs bear a high level of histone H2A/H4 arginine3 symmetrical dimethylation (H2A/H4R3me2s) (Ancelin et al. 2006), a modification conferred by a protein arginine methyltransferase (PRMT) (Bedford and Clarke 2009). Future studies will be needed to elucidate whether the active, passive, or both mechanisms are involved in the genome-wide DNA demethylation in PGCs, and its mechanistic linkage with histone modification reprogramming.

7 PERSPECTIVE

Investigations during the last decade have unveiled key mechanisms underlying PGC development in mice. Continuation of careful studies on the mechanism of germ cell

development and the step-by-step in vitro reconstruction of the information obtained will be major initiatives for the advancement of this field in the next decade. The key function of the germline is to establish totipotency. Toward this end, PGCs first erase epigenetic information inherited from a previous generation, most remarkably as manifested by genome-wide DNA demethylation. PGCs undergo this process while maintaining the underlying potential pluripotency. Subsequently, germ cells gradually establish appropriate epigenetic modifications consistent with embryonic development in a sex-dependent manner. Moreover, in the development of oocytes, they acquire “developmental potency,” which supports the onset of embryonic development. The molecular nature of developmental potency is not well defined. The developmental potency of the oocytes is sufficient to reprogram the epigenome of any somatic cells toward totipotency, although the efficiency is evidently low (Campbell et al. 1996; Wakayama et al. 1998). The mechanism with which the oocytes reprogram somatic nuclei remains poorly understood. Future research should be aimed at understanding the nature of the totipotent epigenome as well as of the developmental potency of the oocytes. Obviously, the studies of the germ cell lineage have wider implications in stem cell biology in general. One of the key issues in stem cell biology is to explore a method to propagate the stem cell state in vitro, without altering its potential to differentiate into a certain lineage. Understanding of the epigenetic regulation of the stem cell state should provide critical information as to how the stem cell state is orchestrated. It is very much hoped that research into the germline, with an emphasis on its epigenetic regulation, will reveal a means of controlling the cells’ epigenetic states in a desirable manner.

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